Lampiran 1. Ethical Clearance

KOMISI ETIK PENELITIAN KESEHATAN (KEPK)
FAKULTAS KEDOKTERAN UNIVERSITAS DIPONEGORO
DAN RSUP dr KARIADI SEMARANG
Sekretariat : Kantor Dekanat FK Undip Lt.3
Jl. Dr. Soetomo 18. Semarang
Telp/Fax. 024-8318350

RSUP Dr. KARIADI

ETHICAL CLEARANCE
No. 26/EC/FK-RSDK/2015

Komisi Etik Penelitian Kesehatan Fakultas Kedokteran Universitas Diponegoro- RSUP. Dr. Kariadi Semarang, setelah membaca dan menelaah Usulan Penelitian dengan judul :

PENGARUH PEMBERIAN EKSTRAK PHALERIA MACROCAPRA TERHADAP SISTEM IMUN PADA MENCIT SWISS DENGAN KARSINOMA EPIDERMOID KULIT (Studi sebagai ajuvant kemoterapi, dengan mengukur ekspresi granzime serta perkembangan massa tumor)

Peneliti Utama	:	**dr. Djoko Handojo, Sp.B, Sp.B(K)Onk**
Anggota Peneliti	:	1. Dr. dr. Selamat Budijitno,M.Si.Med,Sp.B,Sp.B(K)Onk 2. Vito Mahendra Ekasaputra 3. Dimas Erlangga 4. Helmi Sastriawan 5. Yusuf Handoyo 6. Sukri Fitrizan 7. Ferdi Wiweko A 8. Irham Taufiqqurrahman 9. Erwin Suryanegara
Penelitian	:	Dilaksanakan di LPPT Universitas Gadjah Mada, Yogyakarta

Setuju untuk dilaksanakan, dengan memperhatikan prinsip-prinsip yang dinyatakan dalam Deklarasi Helsinki 1975, yang diamended di Seoul 2008 dan Pedoman Nasional Etik Penelitian Kesehatan (PNEPK) Departemen Kesehatan RI 2011

✓ Pada laporan akhir peneliti harus melampirkan cara pemeliharaan & dekapitasi hewan coba dan melaporkan ke KEPK bahwa penelitian sudah selesai dilampiri Abstrak Penelitian.

Semarang, 16 FEB 2015

Komisi Etik Penelitian Kesehatan
Fakultas Kedokteran Undip-RSUP Dr. Kariadi
Ketua

KEPK UNDIP RSUP Dr. KARIADI

Prof. Dr.dr. Suprihati, M.Sc, Sp.THT-KL(K)
NIP.19500621 197703 2 001

Lampiran 2. Hasil Analisis Data Caspase 3 dengan SPSS

Explore

Expresi Caspase 3

Case Summaries

Expresi Caspase 3

Kelompok	N	Mean	Std. Deviation	Median	Minimum	Maximum
K	3	16,533	,4619	16,800	16,0	16,8
P1	3	23,467	1,0263	23,200	22,6	24,6
P2	3	24,400	,3464	24,200	24,2	24,8
P3	3	27,000	,3464	27,200	26,6	27,2
Total	12	22,850	4,0758	24,200	16,0	27,2

Tests of Normality

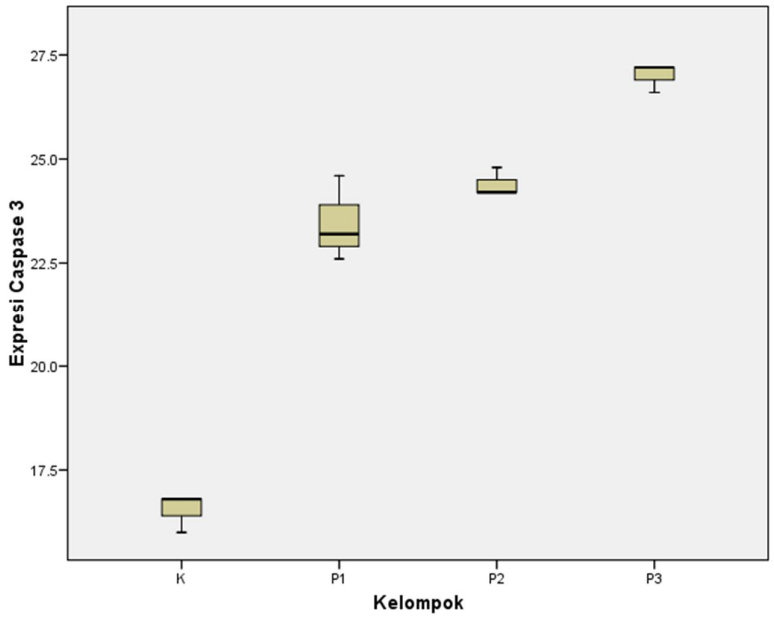
		Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Kelompok	Statistic	df	Sig.	Statistic	df	Sig.
Expresi Caspase 3	K	,385	3	.	,750	3	,000
	P1	,269	3	.	,949	3	,567
	P2	,385	3	.	,750	3	,000
	P3	,385	3	.	,750	3	,000

a. Lilliefors Significance Correction

Test of Homogeneity of Variances

Expresi Caspase 3

Levene Statistic	df1	df2	Sig.
2,643	3	8	,121



NPar Tests

Kruskal-Wallis Test

Ranks

	Kelompok	N	Mean Rank
Expresi Caspase 3	K	3	2,00
	P1	3	5,67
	P2	3	7,33
	P3	3	11,00
	Total	12	

Test Statistics[a,b]

	Expresi Caspase 3
Chi-Square	9,769
df	3
Asymp. Sig.	,021

a. Kruskal Wallis Test

b. Grouping Variable: Kelompok

NPar Tests

Mann-Whitney Test

Ranks

	Kelompok	N	Mean Rank	Sum of Ranks
Expresi Caspase 3	K	3	2,00	6,00
	P1	3	5,00	15,00
	Total	6		

Test Statistics[b]

	Expresi Caspase 3
Mann-Whitney U	,000
Wilcoxon W	6,000
Z	-1,993
Asymp. Sig. (2-tailed)	,046
Exact Sig. [2*(1-tailed Sig.)]	,100[a]

a. Not corrected for ties.

b. Grouping Variable: Kelompok

NPar Tests

Mann-Whitney Test

Ranks

	Kelompok	N	Mean Rank	Sum of Ranks
Expresi Caspase 3	K	3	2,00	6,00
	P2	3	5,00	15,00
	Total	6		

Test Statistics[b]

	Expresi Caspase 3
Mann-Whitney U	,000
Wilcoxon W	6,000
Z	-2,023
Asymp. Sig. (2-tailed)	,043
Exact Sig. [2*(1-tailed Sig.)]	,100[a]

a. Not corrected for ties.

b. Grouping Variable: Kelompok

NPar Tests

Mann-Whitney Test

Ranks

	Kelompok	N	Mean Rank	Sum of Ranks
Expresi Caspase 3	K	3	2,00	6,00
	P3	3	5,00	15,00
	Total	6		

Test Statistics[b]

	Expresi Caspase 3
Mann-Whitney U	,000
Wilcoxon W	6,000
Z	-2,023
Asymp. Sig. (2-tailed)	,043
Exact Sig. [2*(1-tailed Sig.)]	,100[a]

a. Not corrected for ties.

b. Grouping Variable: Kelompok

NPar Tests

Mann-Whitney Test

Ranks

	Kelompok	N	Mean Rank	Sum of Ranks
Expresi Caspase 3	P1	3	2,67	8,00
	P2	3	4,33	13,00
	Total	6		

Test Statistics[b]

	Expresi Caspase 3
Mann-Whitney U	2,000
Wilcoxon W	8,000
Z	-1,107
Asymp. Sig. (2-tailed)	,268
Exact Sig. [2*(1-tailed Sig.)]	,400[a]

a. Not corrected for ties.

b. Grouping Variable: Kelompok

NPar Tests

Mann-Whitney Test

Ranks

	Kelompok	N	Mean Rank	Sum of Ranks
Expresi Caspase 3	P1	3	2,00	6,00
	P3	3	5,00	15,00
	Total	6		

Test Statistics[b]

	Expresi Caspase 3
Mann-Whitney U	,000
Wilcoxon W	6,000
Z	-1,993
Asymp. Sig. (2-tailed)	,046
Exact Sig. [2*(1-tailed Sig.)]	,100[a]

a. Not corrected for ties.

b. Grouping Variable: Kelompok

NPar Tests

Mann-Whitney Test

Ranks

	Kelompok	N	Mean Rank	Sum of Ranks
Expresi Caspase 3	P2	3	2,00	6,00
	P3	3	5,00	15,00
	Total	6		

Test Statistics[b]

	Expresi Caspase 3
Mann-Whitney U	,000
Wilcoxon W	6,000
Z	-2,023
Asymp. Sig. (2-tailed)	,043
Exact Sig. [2*(1-tailed Sig.)]	,100[a]

a. Not corrected for ties.

b. Grouping Variable: Kelompok

Lampiran 3. Hasil Analisis Data Indeks Apoptosis dengan SPSS

Explore

Indeks Apoptosis

Case Summaries

Indeks Apoptosis

Kelompok	N	Mean	Std. Deviation	Median	Minimum	Maximum
K	3	,667	,2309	,800	,4	,8
P1	3	2,333	,5033	2,400	1,8	2,8
P2	3	2,600	,3464	2,800	2,2	2,8
P3	3	3,267	,3055	3,200	3,0	3,6
Total	12	2,217	1,0461	2,600	,4	3,6

Tests of Normality

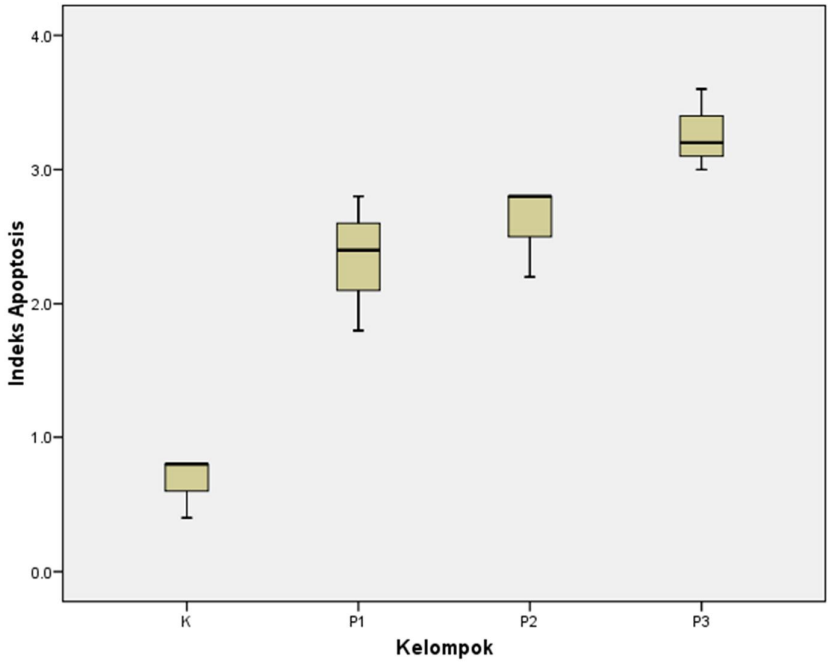
		Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Kelompok	Statistic	df	Sig.	Statistic	df	Sig.
Indeks Apoptosis	K	,385	3	.	,750	3	,000
	P1	,219	3	.	,987	3	,780
	P2	,385	3	.	,750	3	,000
	P3	,253	3	.	,964	3	,637

a. Lilliefors Significance Correction

Test of Homogeneity of Variances

Indeks Apoptosis

Levene Statistic	df1	df2	Sig.
,676	3	8	,591



NPar Tests

Kruskal-Wallis Test

Ranks

	Kelompok	N	Mean Rank
Indeks Apoptosis	K	3	2,00
	P1	3	6,00
	P2	3	7,00
	P3	3	11,00
	Total	12	

Test Statistics[a,b]

	Indeks Apoptosis
Chi-Square	9,630
df	3
Asymp. Sig.	,022

a. Kruskal Wallis Test

b. Grouping Variable: Kelompok

NPar Tests

Mann-Whitney Test

Ranks

	Kelompok	N	Mean Rank	Sum of Ranks
Indeks Apoptosis	K	3	2,00	6,00
	P1	3	5,00	15,00
	Total	6		

Test Statistics[b]

	Indeks Apoptosis
Mann-Whitney U	,000
Wilcoxon W	6,000
Z	-1,993
Asymp. Sig. (2-tailed)	,046
Exact Sig. [2*(1-tailed Sig.)]	,100[a]

a. Not corrected for ties.

b. Grouping Variable: Kelompok

NPar Tests

Mann-Whitney Test

Ranks

	Kelompok	N	Mean Rank	Sum of Ranks
Indeks Apoptosis	K	3	2,00	6,00
	P2	3	5,00	15,00
	Total	6		

Test Statistics[b]

	Indeks Apoptosis
Mann-Whitney U	,000
Wilcoxon W	6,000
Z	-2,023
Asymp. Sig. (2-tailed)	,043
Exact Sig. [2*(1-tailed Sig.)]	,100[a]

a. Not corrected for ties.

b. Grouping Variable: Kelompok

NPar Tests

Mann-Whitney Test

Ranks

	Kelompok	N	Mean Rank	Sum of Ranks
Indeks Apoptosis	K	3	2,00	6,00
	P3	3	5,00	15,00
	Total	6		

Test Statistics[b]

	Indeks Apoptosis
Mann-Whitney U	,000
Wilcoxon W	6,000
Z	-1,993
Asymp. Sig. (2-tailed)	,046
Exact Sig. [2*(1-tailed Sig.)]	,100[a]

a. Not corrected for ties.

b. Grouping Variable: Kelompok

NPar Tests

Mann-Whitney Test

Ranks

	Kelompok	N	Mean Rank	Sum of Ranks
Indeks Apoptosis	P1	3	3,00	9,00
	P2	3	4,00	12,00
	Total	6		

Test Statistics[b]

	Indeks Apoptosis
Mann-Whitney U	3,000
Wilcoxon W	9,000
Z	-,696
Asymp. Sig. (2-tailed)	,487
Exact Sig. [2*(1-tailed Sig.)]	,700[a]

a. Not corrected for ties.

b. Grouping Variable: Kelompok

NPar Tests

Mann-Whitney Test

Ranks

	Kelompok	N	Mean Rank	Sum of Ranks
Indeks Apoptosis	P1	3	2,00	6,00
	P3	3	5,00	15,00
	Total	6		

Test Statistics[b]

	Indeks Apoptosis
Mann-Whitney U	,000
Wilcoxon W	6,000
Z	-1,964
Asymp. Sig. (2-tailed)	,050
Exact Sig. [2*(1-tailed Sig.)]	,100[a]

a. Not corrected for ties.

b. Grouping Variable: Kelompok

NPar Tests

Mann-Whitney Test

Ranks

	Kelompok	N	Mean Rank	Sum of Ranks
Indeks Apoptosis	P2	3	2,00	6,00
	P3	3	5,00	15,00
	Total	6		

Test Statistics[b]

	Indeks Apoptosis
Mann-Whitney U	,000
Wilcoxon W	6,000
Z	-1,993
Asymp. Sig. (2-tailed)	,046
Exact Sig. [2*(1-tailed Sig.)]	,100[a]

a. Not corrected for ties.

b. Grouping Variable: Kelompok

Lampiran 4. Hasil Analisis Korelasi Caspase 3 dan Indeks Apoptosis dengan SPSS

Explore

Case Summaries

	Indeks Apoptosis	Expresi Caspase 3
N	12	12
Mean	2,217	22,850
Std. Deviation	1,0461	4,0758
Median	2,600	24,200
Minimum	,4	16,0
Maximum	3,6	27,2

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Indeks Apoptosis	,211	12	,145	,901	12	,161
Expresi Caspase 3	,226	12	,093	,837	12	,025

a. Lilliefors Significance Correction

Nonparametric Correlations

Correlations

			Indeks Apoptosis	Expresi Caspase 3
Spearman's rho	Indeks Apoptosis	Correlation Coefficient	1,000	,961**
		Sig. (2-tailed)	.	,000
		N	12	12
	Expresi Caspase 3	Correlation Coefficient	,961**	1,000
		Sig. (2-tailed)	,000	.
		N	12	12

**. Correlation is significant at the 0.01 level (2-tailed).

Lampiran 5. Spesifikasi Caspase3 (CPP32) Ab-4

Thermo SCIENTIFIC

DATA SHEET
Rev 0202051

Caspase 3 (CPP32) Ab-4

Rabbit Polyclonal Antibody

Cat. #RB-1197-P0, -P1, or -P (0.1ml, 0.5ml, or 1.0ml at 1.0mg/ml) (Purified Ab with BSA and Azide)
Cat. #RB-1197-P1ABX or -PABX (0.5ml or 1.0ml at 1.0mg/ml) (Purified Ab without BSA and Azide)
Cat. #RB-1197-B0, -B1, or -B (0.1ml, 0.5ml, or 1.0ml at 1.0mg/ml) (Biotin-labeled Ab with BSA and Azide)
Cat. #RB-1197-R7 (7 ml) (Ready-To –Use for Immunohistology)
Cat. #RB-1197-PCS (5 Slides) (Positive Control for Histology)
Cat. #RB-1197-PCL (0.1ml) (Positive Control for Western Blot)

Description: Caspase 3 is ubiquitously expressed and like other caspases is synthesized as an inactive (32kDa) proenzyme. Upon activation, Caspase 3 is cleaved at Asp28-Ser29 and Asp175-Ser176 thereby generating two subunits of 17kDa and 12kDa, respectively. Recent studies have implicated that Caspase 3 is associated with the induction of apoptosis. Activation of Caspase 3 occurs in response to variety of apoptotic inducers including Fas mediated apoptosis.

Mol. Wt. of Antigen: 32kDa

Epitope: Not determined

Species Reactivity: Human, Mouse, Rat, Monkey, Rabbit, Hamster, Cow, Sheep, Pig, Dog. Others-not tested.

Immunogen: Recombinant full length human caspase 3 protein.

Applications and Suggested Dilutions:

- Western Blotting (Ab 2.5-5µg/ml for 2hrs at RT)
- Immunoprecipitation (Denatured verified) (Use Protein A) (Ab 10µg/mg protein lysate)
- Immunohistology (Formalin/paraffin) (Ab 5-10µg/ml for 30 min at RT; Ab-3 is better)

* [Staining of formalin-fixed tissues REQUIRES boiling tissue sections in 10mM citrate buffer, pH 6.0, (***NEOMARKERS'*** Cat. #AP-9003), for 10-20 min followed by cooling at RT for 20 min.]

The optimal dilution for a specific application should be determined by the investigator.

Positive Control: Jurkat cells. Tonsil.

Cellular Localization: Predominantly Cytoplasmic with some nuclear staining

Supplied As:
Total IgG purified from rabbit anti-serum by Protein A chromatography. Prepared at 1mg/ml in 10mM PBS, pH 7.4, with 0.2% BSA & 0.09% sodium azide. Also available without BSA and azide at 1mg/ml.

Storage and Stability:
Ab with sodium azide is stable for 24 months when stored at 2-8°C. Antibody WITHOUT sodium azide is stable for 36 months when stored at below 0°C.

Suggested References:

1. Krajewska M, et al. Cancer Res 1997, 57(8):1605-13.
2. Mallat Z, et al. Circulation 1997, 96(2):424-8.

Limitations and Warranty:
Our products are intended FOR RESEARCH USE ONLY and are not approved for clinical diagnosis, drug use or therapeutic procedures. No products are to be construed as a recommendation for use in violation of any patents. We make no representations, warranties or assurances as to the accuracy or completeness of information provided on our data sheets and website. Our warranty is limited to the actual price paid for the product. NeoMarkers is not liable for any property damage, personal injury, time or effort or economic loss caused by our products.

Material Safety Data:
This product is not licensed or approved for administration to humans or to animals other than the experimental animals. Standard Laboratory Practices should be followed when handling this material. The chemical, physical, and toxicological properties of this material have not been thoroughly investigated. Appropriate measures should be taken to avoid skin and eye contact, inhalation, and ingestion. The material contains 0.09% sodium azide as a preservative. Although the quantity of azide is very small, appropriate care should be taken when handling this material as indicated above. The National Institute of Occupational Safety and Health has issued a bulletin citing the potential explosion hazard due to the reaction of sodium azide with copper, lead, brass, or solder in the plumbing systems. Sodium azide forms hydrazoic acid in acidic conditions and should be discarded in a large volume of running water to avoid deposits forming in metal drainage pipes.

For Research Use Only

Thermo Fisher Scientific
Anatomical Pathology
46360 Fremont Blvd.
Fremont, CA 94538, USA
Tel: 1-510-771-1560
Fax: 1-510-771-1570
http://www.thermo.com/labvision

Manufactured by:
NeoMarkers
For
Lab Vision Corporation

EC REP

Thermo Fisher Scientific
Anatomical Pathology
93-96 Chadwick Road, Astmoor
Runcorn, Cheshire WA7 1PR, UK
Tel: 44-1928-562600
Fax: 44-1928-562627
Labvision.uk@thermofisher.com

Lampiran 6. Cara pemeliharaan dan dekapitasi hewan coba

A. Cara pemeliharaan

Sejumlah 24 mencit Swiss diadaptasi selama satu minggu, diberi makanan dan minuman dalam jumlah yang sama, kemudian dilakukan induksi tumor. Penelitian ini menggunakan sampel 24 ekor mencit jantan strain Swiss, setelah diadaptasi selama 7 hari tidak didapatkan mencit yang sakit maupun mati. Setelah itu dilakukan induksi sel karsinoma epidermoid terhadap mencit dengan cara mengoleskan 9, 12-dimethyl-1,2-benzanthracene (DMBA) dan 12-o-tetradecanoylphorbol-13-acetate (TPA). Evaluasi induksi selama 9 bulan didapatkan 18 ekor mencit berhasil tumbuh tumor pada bagian kulit yang diinduksi. Randomisasi dilakukan dengan cara penomoran dan pengundian ke dalam 4 kelompok dan ditempatkan dalam 4 kandang terpisah.

B. Cara dekapitasi

Dekapitasi dilakukan dalam anesthesia dengan ether. Kapas yang telah dituangi 10-20 ml ether dimasukkan ke dalam sebuah wadah yang transparan, kemudian mencit dimasukkan ke dalam wadah tersebut dan ditutup rapat. Dilakukan pengamatan terhadap pernafasan mencit. Setelah nafas berhenti, mencit dikeluarkan dari wadah dan dilakukan dislokasi pada vertebra cervivalnya dengan menggunakan gunting. Setelah itu dilakukan pembedahan untuk mengambil tumor.

Lampiran 7. Dokumentasi penelitian

Foto mencit swiss yang sedang diinduksi karsinoma epidermoid

Foto mencit swiss yang sudah tumbuh tumor, dibagi dalam kelompok perlakuan

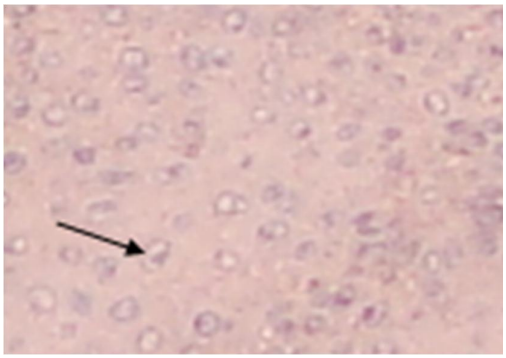
Foto mencit swiss saat dilakukan pemberian kemoterapi

Foto mencit swiss saat dilakukan terminasi

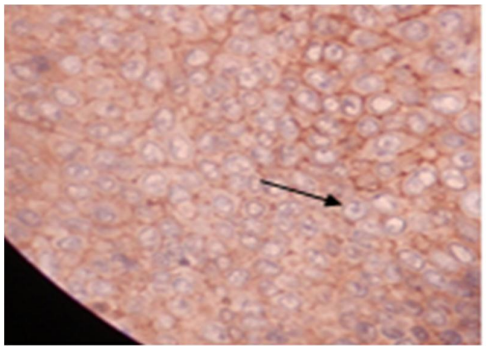
Foto pembuatan preparat histology

Foto pembacaan preparat hitologi

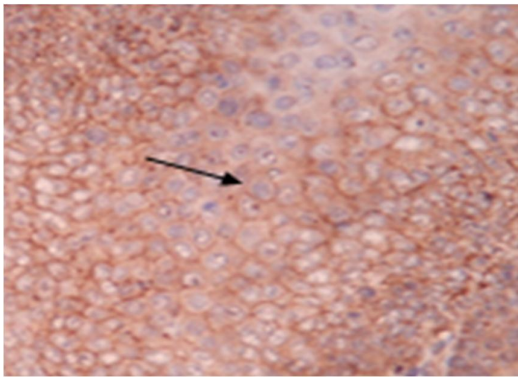
Hasil pemeriksaan histologi



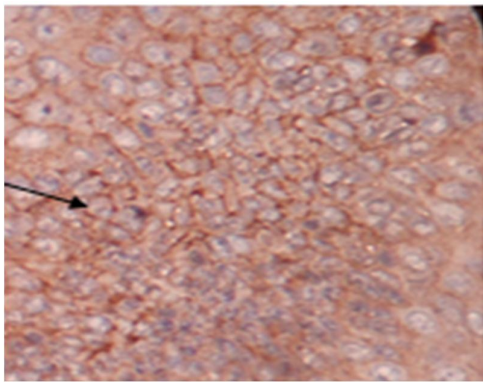
Gambaran histologis ekspresi caspase 3 (panah), kelompok Kontrol (K)



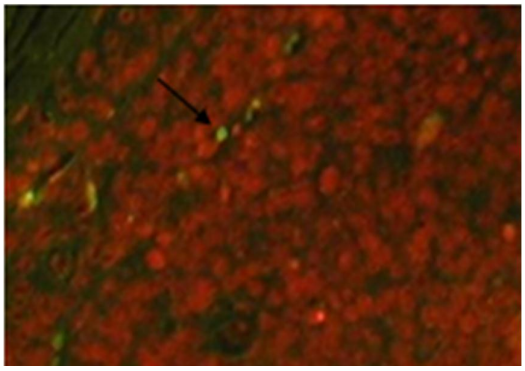
Gambaran histologis ekspresi caspase 3 (panah), kelompok P1

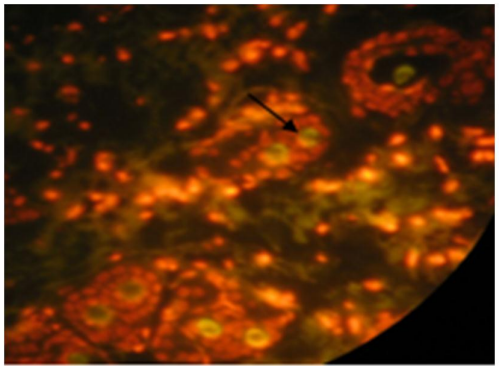


Gambaran histologis ekspresi caspase 3 (panah), kelompok P2

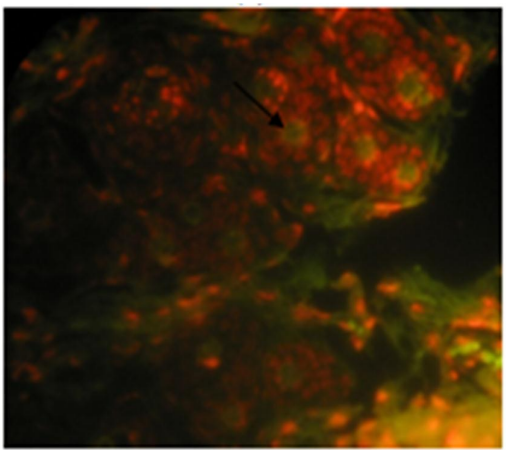


Gambaran histologis ekspresi caspase 3 (panah) kelompok P3

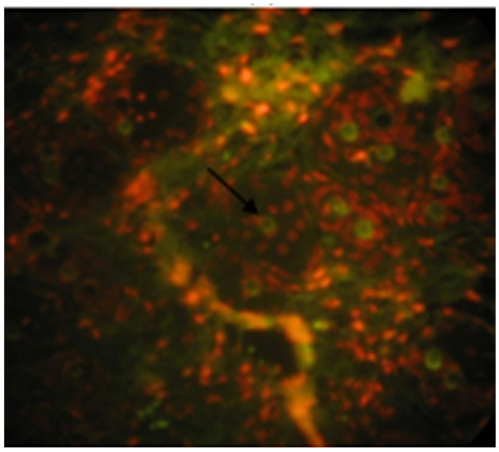




Gambaran histologis apoptosis (panah), kelompok Kontrol



Gambaran histologis apoptosis (panah), kelompok P1



Gambaran histologis apoptosis (panah), kelompok P2

Gambaran histologis apoptosis (panah), kelompok P3

Data Hasil Penelitian

Kelompok	Hasil Pemeriksaan Histologi	Ekspresi Caspase 3 Perseratus sel					Jumlah	Mean
		Lapanga pandang						
		I	II	III	IV	V		
IA	SCC Well Diff	13	17	17	18	15	80	16
IB	SCC Insitu	17	14	16	18	19	84	16.8
IC	SCC Insitu	19	17	20	13	15	84	16.8
IIIA	Keratoaantoma dengan SCC Mikroinvasif	23	21	24	22	23	113	22.6
IIIB	Papiloma Cell Squamosa dengan fokus Ca	24	22	27	24	26	114	24.6
IIIC	SCC Insitu	24	23	24	22	23	116	23.2
VA	SCC Well Diff dengan nekrosis luas	27	22	26	23	23	121	24.2
VB	SCC Well Diff	23	23	22	26	27	121	24.2
VC	SCC Well Diff	23	26	24	29	22	124	24.8
VIA	SCC Well Diff	25	24	30	26	28	133	26.6
VIB	SCC Insitu	29	26	23	31	27	136	27.2
VID	SCC Insitu dengan Mikroinvasif	23	31	25	28	29	136	27.2

Kelompok	Hasil Pemeriksaan Histologi	Indeks Apoptosis Perseratus sel					Jumlah	Mean
		Lapanga pandang						
		I	II	III	IV	V		
IA	SCC Well Diff	0	0	1	1	0	2	0.4
IB	SCC Insitu	1	2	1	0	0	4	0.8
IC	SCC Insitu	2	0	0	1	1	4	0.8
IIIA	Keratoaantoma dengan SCC Mikroinvasif	2	5	3	2	0	12	2.4
IIIB	Papiloma Cell Squamosa dengan fokus Ca	3	2	4	1	4	14	2.8
IIIC	SCC Insitu	4	1	2	1	1	11	1.8
VA	SCC Well Diff dengan nekrosis luas	1	3	4	1	2	11	2.2
VB	SCC Well Diff	2	3	1	4	4	14	2.8
VC	SCC Well Diff	4	2	3	2	3	15	2.8
VIA	SCC Well Diff	4	4	3	1	3	15	3
VIB	SCC Insitu	3	4	2	4	3	16	3.2
VID	SCC Insitu dengan Mikroinvasif	2	2	5	5	4	18	3.6

UNIVERSITAS GADJAH MADA
LABORATORIUM PENELITIAN DAN PENGUJIAN TERP
(LPPT – UGM)
Bidang Layanan Penelitian Pra – Klinik dan Pengembangan Hewan Per
Jl. Agro Karang Malang Kampus UGM
Telp. (0274) 7497705, FAX. (0274) 546868, e-mail: lppt_info@mail.ugm.ac.

SURAT KETERANGAN
NO : 696/LP3HP/23 - VI/2014

Bersama ini kami menerangkan bahwa ;

Nama	:	dr. Helmi Sastriawan
NIM	:	22010110400059
Instansi	:	Program Magister Ilmu Biomedik Fakultas Kedokteran Universitas Diponegoro Semarang.
Jenjang Studi	:	S2

Benar – benar telah selesai melakukan Penelitian di Unit Layanan Penelitian Pra – Klinik dan pengembangan Hewan Percobaan (LP3HP) LPPT UGM. Pada bulan Mei 2014 sesuai Proposal yang diajukan dengan judul :

"PERBANDINGAN EKSPRESI ENZIM CASPASE-3 AKTIF DAN INDEKS SEL APOPTOSIS PADA MENCIT DENGAN CA EPIDERMOID YANG DIBERIKAN EKSTRAK *Phaleria macrocarpa* SITOSTATIKA DAN KOMBINASI *Phaleria macrocarpa* DAN SITOSTATIKA"

Dan dinyatakan bebas dari segala tanggungan di Laboratorium Penelitian dan Pengujian Terpadu Universitas Gadjah Mada.

Demikian surat keterangan ini dibuat, agar dapat dipergunakan sebagaimana mestinya.

Atas kerjasama yang baik diucapkan banyak terima kasih

Yogyakarta, 24 Juni 2014
PLT. Kabid Unit Pra- Klinik LPPT UGM

Drh. Claude Mona Airin, MP.
NIP : 19760708 200801 2 012